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RELATIONSHIPS OF THE CARDIAC SIGNAL AND HEAT ACCLIMATED STATE: SPECTRAL PROFILES FROM ECG ANALYSIS

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13. ABSTRACT *(Maximum 200 words)*
ECG signal has been analyzed to assess the state of heat acclimation in rats. Adult rats, were divided into control and acclimation groups. The ECG signals were obtained from the telemetry transmitters implanted into the rats. The power spectral density of unit time-differenced ECG series was computed. The spectral profiles for the acclimated rats show significantly smoother profiles following heat acclimation compared to the same for the control rats. The progression of acclimation also can be confirmed from the statistical comparison against the acclimated spectral pattern. The Mann-Whitney rank sum statistic (MWRS) between the average energy spectral profile and the spectral profile for individual days shows that subsequent to acclimation, the MWRS usually drops to low values (usually <1), whereas it remains >1 and usually >1.96 for the control group. The differential profiles derived by subtracting the bi-directionally low-pass filtered profile from the original spectral profile show distinctly reduced variations for the heat acclimated rats compared to the control rats.

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EXECUTIVE SUMMARY

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INTRODUCTION

Variability exists in individual tolerance to high environmental temperatures. Intolerant individuals are more likely to suffer heat exhaustion or heat stroke during exposure to high ambient temperatures or while working in more moderate temperatures [2]. Repeated exposure to a hot environment elicits physiological adaptations that increase tolerance to heat stress. When tolerance occurs as a result of protracted residence in a natural hot climate, it is termed acclimatization. A similar physiological state can be achieved under experimental conditions during acute exposure to a hot environment for 5 or more days and is termed acclimation.

While humans rely on the evaporation of sweat for evaporative cooling, rats spread saliva over their ventral surfaces for evaporative cooling when they become hyperthermic [5]. Although there is individual variability in heat tolerance and ability to acclimate, the following are among the physiological changes generally observed with acclimation in both man [16] and rats [4, 6, 7, 8] There is an increase in plasma volume, tolerance time in the heat, skin blood flow, time interval during which sweating (saliva spreading in rats) can be maintained; there is a decrease in basal heart rate (HR) and HR during heat exposure, core temperature (Tc) during heat exposure, and Tc threshold for sweating (in terms of salivation).

The autonomic nervous system plays a central role in the modulation of heart rate during thermoregulation and acclimation. In anesthetized rats, heart rate increases with increasing Tc, and this modulation is a result of interaction between sympathetic and parasympathetic input [7,15]. This progressive increase in heart rate with increasing Tc results from a combination of increased firing rate of intrinsic cardiac pacemaker cells and the effect of the combination of decreasing parasympathetic and increasing sympathetic input [15]. During the course of heat acclimation bradycardia develops beginning as early as the second day. The changes in heart rate through the course of acclimation is a dynamic process with early decreases being mediated largely by increased parasympathetic and decreased sympathetic input. Later (beyond 2 weeks) in the course of acclimation, intrinsic heart rate decreases and the role of the autonomic nervous system although still significant may play less of a role in maintaining the bradycardia [7]. Animals subjected to a heat stress test following acclimation have a significantly attenuated tachycardia compared to unacclimated control animals [7,13].

Heart rate variability (HRV) or the R-R interval lengths (RRI) (of consecutive QRS complexes in the ECG signal) are often analyzed to assess the cardiac condition [11, 14]. Reduction in HRV in the fetus during labor has been recognized as a sign of fetal distress since the 60's [1]. Additionally, evidence of an association between lethal arrhythmias and an increase in sympathetic or decrease in vagal activity has stimulated efforts to identify quantifiable markers of this autonomic activity. Analysis of HRV variation allows deductions on the state and function of central nervous oscillation, sympathetic and efferent vagal activity, humeral effects, and the sinus node activity [11].

The conventional HRV or RRI studies are based on spectral analysis [12]. The main emphasis is to determine the comparative signal strengths in different frequency ranges and to correlate the same with the functional activities within the physiological system.

In the present work, instead of HRV or RRI series, the ECG signal itself has been analyzed to assess the relationship between the cardiac signal and the state of heat acclimation. Data for this analysis were archived data drawn from a previously published study of heat acclimation in telemetry-equipped rats [13].

The objective of the present work was to determine, if correlation exists between the state of heat acclimation and the cardiovascular condition as detected by the ECG signal, and whether such a relationship can be detected from the characteristics of short segments of ECG data.

METHODS

EXPERIMENTAL SETUPS

Adult male Sprague-Dawley rats used for the present study were fitted with surgically implanted sensor-transmitters (Data Sciences, St. Paul, Minnesota, Cat #TL11M2-C50-PXT), measuring physical activity, ECG, blood pressure, core temperature (Tc) and heart rate (HR). The data were recorded using a PC and DataQuest IV software. The details of the experimental setup as well as the HR and BP data are contained in Matthew (1997) [13].

The rats were divided into two groups: control and acclimation. Although there were 6 rats in each group in the original study [13], ECG data were retrievable for only 5 animals in each group. Following 2 weeks of recovery from implantation surgery, both groups were housed at 26°C, 50% rh for pre-acclimation data collection. The control rats were maintained at 26°C, 50% rh, and the rats belonging to the acclimation group were subsequently housed at 32-33°C, 50% rh for the 2 week acclimation period. For the present study, data collected over six recording days (spanning over 12-13 days) were used.

ECG waveforms were collected at the same time of day, 3 days per week prior to and through the 2 weeks of acclimation. Ten second strips were taken every 10 minutes at a sampling frequency of 1000 Hz. The core temperature (Tc), blood pressure, and heart rate data were recorded once every 10 minutes.

SPECTRAL ANALYSIS

The power spectra of the recorded ECG data were produced using Welch's method [12]. Successive overlapping segments of unit time-differenced data were considered. The unit time differencing blocks the steady-state or the zero-frequency information associated with the series. For the power spectral decomposition 2048

point long data sets were used with Hanning windows of length 256, and a data-overlapping window of length 128.

ASSESSMENT OF HEAT ACCLIMATION

The prime acclimation information lies within the lower segment of the spectral profile, while the influence of the extraneous noise can be spread over the entire spectrum. So, for the assessment of the acclimation state, the segment of the spectral pattern over 300Hz is considered. To get an overall idea of the dynamic process, state-space plots corresponding to each spectral distribution (over 0-300 Hz) are also studied. While the spectral pattern itself may convey visual assessment of the acclimation state, a quantified assessment of the degree of acclimation attained is derived using the Mann-Whitney rank sum statistic [17]. The null hypothesis is that two sets of normally distributed data are considered to come from the same distribution; if the Mann-Whitney rank sum statistic $|Z|>1.96$, the null hypothesis can be rejected with greater than 95% confidence level [17].

THE AVERAGE ENERGY PATTERN

Consider cyclical data series $\{x(k)\}$ with fixed periodicity N being arranged in successive rows of a matrix A. The average energy pattern across the rows can be computed as follows. The singular value decomposition [16,17] of A is given by $A_N = USV^T$, where $U \in R^{m\times m}$ and $V \in R^{N\times N}$ are orthogonal matrices, $S\ (R^{m\times m}) = \mathrm{diag}\ (s_1, s_2, \ldots, s_r : 0)$, $r = \min(m,N)$, $s_1 \geq s_2 \geq \ldots \geq s_r$. The best rank-1 approximation of the periodic component of periodicity N in $\{x(k)\}$ in least squares sense is given by (the time series represented by) $u_1 s_1 v_1^T$, where u_1 and v_1 are the first columns of U and V respectively; v_1^T represents the pattern over the periodic segments of the extracted component of periodicity N, while the successive elements of $u_1 s_1$ are the scaling factors for the successive periodic segments. Since $u_1^T u_1 = 1$, and u_1 is m-long, the average energy pattern will be given by $s_1 v_1^T/(m)^{1/2}$ [9].

In the present case, the spectral profiles for the unit differenced ECG data for a particular recorded day for all the five acclimation-rats are arranged in the successive rows of a matrix. Since the prime information lies in the first 300 data points in each case, these points only are used to compute the average energy pattern. The progression of the acclimation is now analyzed using these patterns. The average energy patterns are also used to compute the comparative Mann Whitney rank-sum statistic against the individual profiles for each rat.

BI-DIRECTIONAL FILTERING

As the state of heat acclimation tends to be reflected in variations in the spectral profiles although individual variability exists, the difference between the spectral profile and the bi-directional filtered profile may be of interest to reduce the element of variability of the spectral profiles. Bi-directional filtering [9] is appropriate in the present context as here the data-series passed through the same filter in the forward and in the

reverse directions to eliminate the generated phase-shift. A single pole filter with a pole at 0.8 was used. The differenced profiles are studied for both the control and the acclimation group of rats.

RESULTS AND DISCUSSION

Figure 1(a) and (b) show the typical ECG patterns for a rat on a pre-acclimation day and on a day, when the rat is exposed to elevated temperature for heat acclimation. The schedule for the exposure to heat for acclimation is mentioned in Table 1.

STUDY 1: SPECTRAL PROFILES AND STATE-SPACE-PLOTS:

Figures 2(a) to 2(f) show the spectral profiles of the acclimation rat 950059, and the corresponding state-space plots. Figures 3 to 6 show the spectral profiles and the state-space plots for the other acclimation rats 950158, 960017, 960034 and 960107.

Figures 7 to 11 show the spectral profiles and the state-space plots results for the control rats 950067, 950160, 960015, 960033, 960093.

The observations can be summarized as follows:

(1) Both in Figure 2 and Figure 3, both in the spectral profiles and the state-space plots, distinct differences are observed between the acclimation days and the preacclimation days. Subsequent to acclimation the profiles tend to be more uniform with decreased degree of undulations; the state-space plot shows relatively smooth closed contours. For the unacclimated animals, the state-space contours are either not closed or not smooth. The degree of dynamics for both preacclimation days is similar for both rats 950059 and 950158.

(2) Figures 4, and 5 also show distinctly different spectral profile and state-space plot for the preacclimation day and the acclimation days.

(3) The state-space patterns for a rat during the progression of the process of heat acclimation can be different. For example, see the patterns for rat 960107 in Figure 6. Clearly the animal does not reach acclimation state on the second day, as expected. The process of acclimation gradually progresses through the third day and the fourth day but a truly rounded state-space profile is obtained on the fifth day, when the acclimation state can be considered to have been reached.

(4) Figures 7 to 11 show no significant change in the dynamics of the control rats as exhibited in the spectral profiles or the state-space plots across the six recording days, while the animals remain exposed to the same temperatures. The observed undulations in the profiles and the state-space plots are much larger compared to those for the acclimated animals.

Remarks

1. The distinction between the spectral profiles for the acclimated and the unacclimated animals is conspicuous in the state-space representations of the spectral

profiles. The acclimated condition exhibits distinctly rounded off closed contours compared to the unacclimated conditions, where the contours are either sharper or not uniformly closed.

2. The rate of progression towards the steady state condition of heat acclimation under similar thermal exposure can be different in different animals; the time to acclimate too can be different under similar conditions.

3. The variation between the successive days in acclimated or unacclimated conditions is due to the heart rate variability in healthy animals. The reduced variations in the spectra with the progression of acclimation is possibly indicative of thermal stress on the cardiovascular system. The absence of such variations in the control animals indicates the absence of similar stress.

4. The spectral profile is a function of the heart rate, as well as the ECG pattern, and that way it can offer a more complete picture of the acclimation state compared to the assessment using the heart rate alone.

Unlike the acclimated rats, the spectral profiles for the control rats show no significant differences between Day 1 and Day 6, although the patterns are not exactly repetitive, and there is individual variability. Broadly, the natures of the spectra show conspicuously larger variations for the control rats compared to the acclimation rats.

STUDY 2: MANN WHITNEY RANK SUM STATISTIC ASSESSMENT ACROSS THE SPECTRAL PROFILES

The basic idea for this part of the study is to explore if any template spectral profile pattern can be considered to be representative of the acclimation state. We consider the average energy pattern for the acclimation rats for the fourth day and for the fifth day of recording. Figure 12 shows the patterns for the acclimation rats for the sixth day and the corresponding average energy pattern.

Note that in general, the spectral profile corresponding to the acclimation state is expected to exhibit low values of the statistic; a low value of statistic is obtained when the difference between the profile for the average energy pattern and that for the candidate day is minimal.

For statistical comparison between these profiles and the individual profiles through all the six recording days for the individual rats, only the most informative part of the profiles are considered. From empirical analysis, it is decided to consider the segment of the central part of the average energy profile where the magnitude is larger than 15% of the maximum, discarding the rest. The corresponding length of the spectral profiles for all the individual cases are marked and the Mann Whitney statistic is computed. The results for the fourth day and the fifth day are presented in Table 2 and Table 3 respectively.

The following observations deserve attention.

(1) In general the results shows low values of the statistic for the acclimation states.

(2) There is a correspondence between the pre-acclimation day value of the statistic and the undulations in the spectral profile, for example note the preacclimation day results for rat 960034 and 960107.

(3) Rats 950059 and 950158 unexpectedly show low values of the statistic even on the two preacclimation days. This can again be ascribed to the nature of the corresponding spectral profiles (see Figures 2 and 3), which are not too jittery, unlike other animals.

(4) For both rat 960107 and 960034, even on the first day of acclimation, relatively high value of statistic is observed. This is due to the fact that the animals were slow to acclimate, which is also revealed in their spectral profiles and state-space plots (Figure 5 and Figure 6).

(5) The case for rat 960017 is different from the last case. As shown in Figure 12, the overall spectral pattern for rat 960017 is different from the other acclimation rats; the response is confined more to relatively lower frequencies, with the peak appearing between 42 Hz for day 2 to 62 Hz for day 6, whereas the average energy profile has peak at 73 Hz.

(6) Note the values appearing in Day 4 column in Table 2 and the Day 5 column in Table 3. All the values of $|Z|$ are less than 1. So, irrespective of the individual variability across the group of rats and the variability during the progression of the process of acclimation for the same rat, the statistic produced by the present study reveals closeness to the average energy pattern, indicating reaching of the state of acclimation.

So, we conclude that in general, the acclimation states exhibit low values of the statistic; a low value of statistic is obtained when the difference between the profile for the sixth day and that for the candidate day is minimal. In statistical terms, $|Z| > 1.96$ indicates the samples constituting the two distributions being drawn from two different experiments. Thus in the present case the fact that $|Z|$ remaining low is related to the acclimation condition, which is confirmed by the results for the acclimation rats. Since for the control rats, there is no standard profile to compare with, MWRS statistic is computed between the profiles for the successive days.

Remarks

1. The question arises whether the average energy pattern can be considered as the expected pattern for all the rats undergoing heat acclimation. Our study shows that it is difficult to arrive at one specific heat-acclimation pattern for the spectral profile for all the rats. The average energy pattern can serve as the broad nature of the expected pattern following heat acclimation, but the individual variability will remain.

2. If the spectral profile is weakly revealing, the consequent Mann Whitney statistic too may not be decisive.

STUDY 3: BI-DIRECTIONALLY FILTERED SPECTRAL PROFILE

Since the unacclimated condition tends to show more undulations in the spectral profile, in this part of the study the difference between the spectral distribution and a bi-directionally filtered version of the spectral profile was computed. For the bi-directional filter, a first order pole of 0.8 was considered. The results for the acclimation rat 960017 and the control rat 960015 are shown in Figures 13 and 14 respectively. Note that the acclimated conditions tend to show less variation in the differenced profiles beyond 200Hz.

STUDY 4: HEART RATES

The heart rates for the control and acclimation groups are given in Tables 3a and 3b. In these tables the pre-acclimation value for those rats for which 2 pre-acclimation days were analyzed is the mean of the 2 values. The individual values for these days are as follows: control 950067- 335 and 324 bpm, control 950160- 353 and 362 bpm, acclimation 950059- 316 and 324 bpm, and acclimation 950158- 295 and 328 bpm. The heart rates of the control group did not change during the course of the 2 weeks of the study. However, the heart rate of the acclimation group decreased from pre-acclimation to acclimation 1 (2 days of heat acclimation), and there was a further decrease from accl 1 to acclimation 2 (4-5 days of acclimation); after accl 2 there was no further decrease. The "t" test values in Table 3b indicate that the pre-acclimation heart rates were not different between the 2 groups, but there was a significant difference between the groups on all days of acclimation.

Remarks

The decrease in the heart rate is expected in response to residence in an elevated ambient temperature. Note that from the heart rate alone, no definite pattern emerges regarding the progression of heat acclimation, unlike the state-space diagrams derived from the spectral profiles.

CONCLUSIONS

As previously reported [13], the acclimated rats in this study exhibited: lower HR during acclimation and throughout a subsequent heat stress, lower Tc during the heat stress test, and lower systolic blood pressure during the heat stress than pair-wise heat stressed control rats. Therefore, the acclimation group in the present study exhibited classical sign of acclimation. The present preliminary study shows that the spectral profiles derived from the ECG signal can be an alternative approach for assessing the heat acclimation state of a rat.

Horowitz and Meiri [7] have previously demonstrated that the decreased HR seen with heat acclimation is in part a function of acceleration of parasympathetic and withdrawal of sympathetic nervous input. In previous work with spectral analysis of HRV, high frequency peaks and the power represented by the area under the curve of the high frequency peak have been used as an indication of parasympathetic activity, and the ratio of low to high frequency power has been used as an indication of sympathetic activity [1, 11]. Therefore, the difference in ECG waveforms seen in this study between control and acclimated rats most likely represents changes in the tone of the autonomic nervous system through the acclimation process. It is necessary to confirm these findings with longer sets of data on acclimated and control rats and to determine whether similar results can be seen in human volunteers.

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Table 1: Heat acclimation record of the rats

Heat Acclimation Record						
Rat No.	Recording Day 1	Recording Day 2	Recording Day 3	Recording Day 4	Recording Day 5	Recording Day 6
950059	Preacclim	Preacclimation	Acclimation	Acclimation	Acclimation	Acclimation
950158	Preacclim	Preacclimation	Acclimation	Acclimation	Acclimation	Acclimation
960017	Preacclim	Preacclimation	Acclimation	Acclimation	Acclimation	Acclimation
960034	Preacclim	Preacclimation	Acclimation	Acclimation	Acclimation	Acclimation
960107	Preacclim	Preacclimation	Acclimation	Acclimation	Acclimation	Acclimation

Table 2a: Statistical Comparison between Average Energy spectral profile for the fourth day and the individual spectral profiles of acclimation rats

Mann Whitney Rank Sum Statistic						
Rat No.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
960017	1.9968	1.8937	2.1970	1.0712	1.0435	1.5548
960034	3.4915	0.9543	0.2923	0.4449	0.4152	0.4370
960107	6.9238	3.08680	0.3597	0.0684	0.1100	0.7442
950059	0.1140	0.1417	0.3082	0.2943	0.1377	0.0307
950158	0.1774	0.2963	0.4370	0.2983	0.1714	0.6748

Table 2b: Statistical Comparison between Average Energy spectral profile for the fifth day and the individual spectral profiles of acclimation rats

Mann Whitney Rank Sum Statistic						
Rat No.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
960017	1.7807	1.6858	1.6593	0.9262	0.8732	1.2420
960034	3.6950	1.4429	0.9152	0.8843	0.2616	0.1733
960107	7.3778	3.7568	0.7452	0.9395	0.3433	0.6767
950059	0.1557	0.1071	0.3279	0.3765	0.3345	0.0320
950158	0.0718	0.4626	0.3875	0.1159	0.1579	0.6789

Table 3a: Heart Rates of Control Rats from Pre-acclimation through Days Corresponding to Acclimation days in the Acclimation Group

Control Rats						
Rat No.	pre-acc I	c-accl 1*	c-accl 2	c-accl 3	c-accl 4	c-accl 5
950067	329	308	344	350	367	341
950160	358	359	345	334	368	357
960015	301	304	292	283	288	305
960033	348	316	340	307	327	307
960093	336	344	356	374	366	346
mean	334	326	335	330	343	331
SD	22	24	25	36	35	24

* control day corresponding to acclimation day 1.

Table 3b: Heart Rates of Acclimated Rats from Pre-acclimation through 2 weeks of Heat Acclimation

Acclimation Rats						
Rat No.	pre-acc I	accl 1	accl 2	accl 3	accl 4	accl 5
950059	320	284	262	259	271	277
950158	311	317	269	281	273	255
960017	333	292	266	289	275	268
960034	330	286	269	276	268	254
960107	345	294	293	281	267	306
Mean	328	295	272	277	271	272
SD	13	13	12	11	3	21
P value*	0.575	0.033	0.001	0.014	0.002	0.003

P value from Student's "t" test comparing the control and acclimation groups. P<0.05 indicates the values are significantly different.

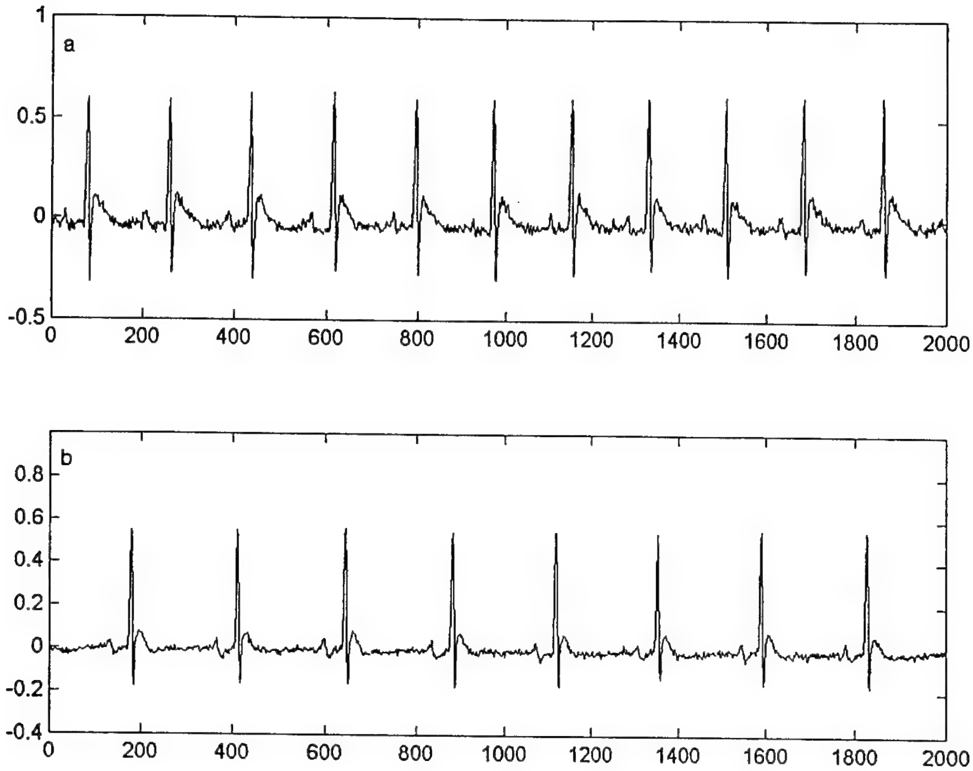

Figure 1 (a) Typical ECG signal for a rat belonging to the Control Group.
(b) Typical ECG signal for a rat belonging to the Acclimation Group on the sixth recorded day in the acclimated state.

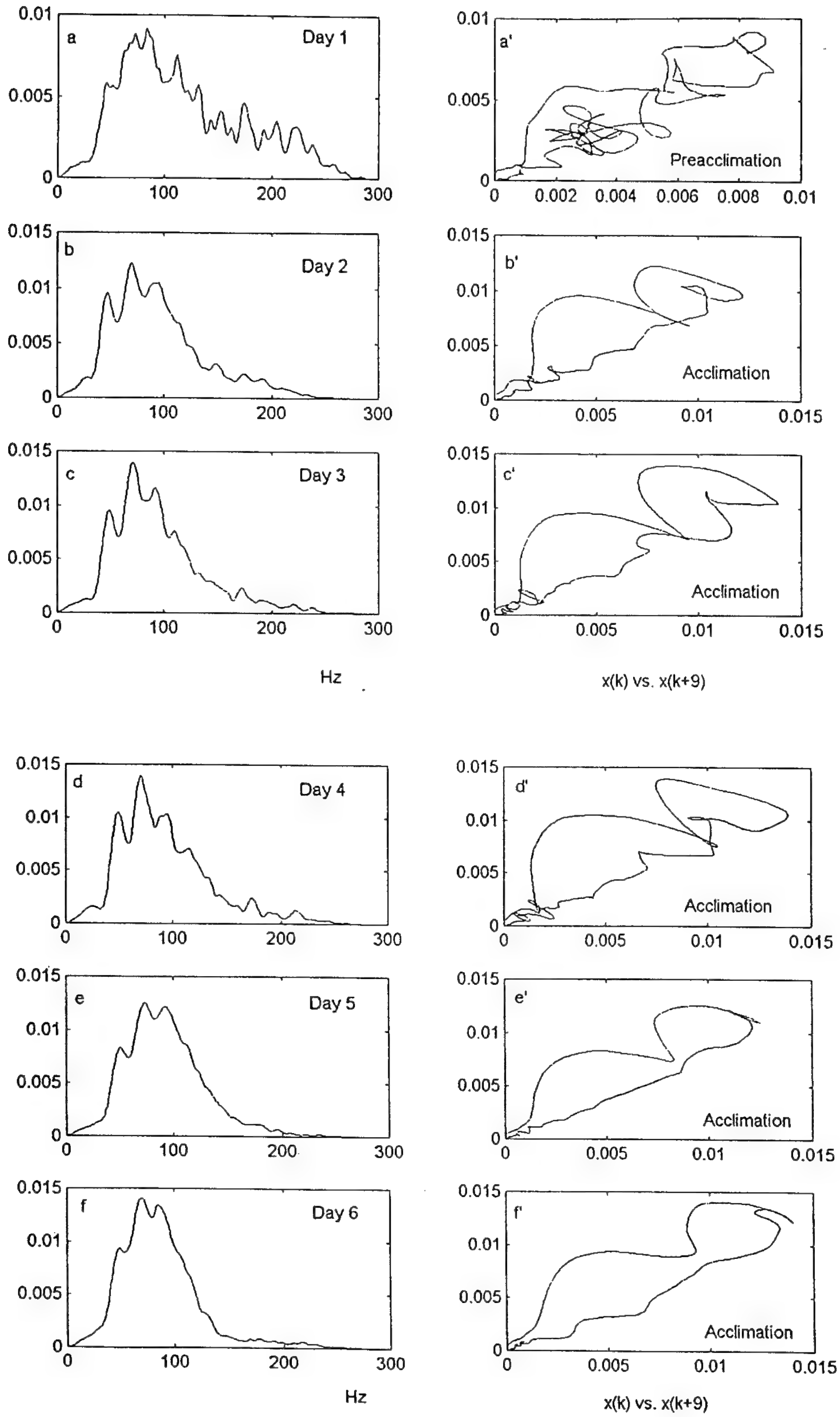

Figure 2 (a) - (f) show the spectral profiles for Rat 960034 from recorded day 1 to day 6. (a') - (f') show the corresponding state-space plots ($x(k)$ vs. $x(k+9)$).

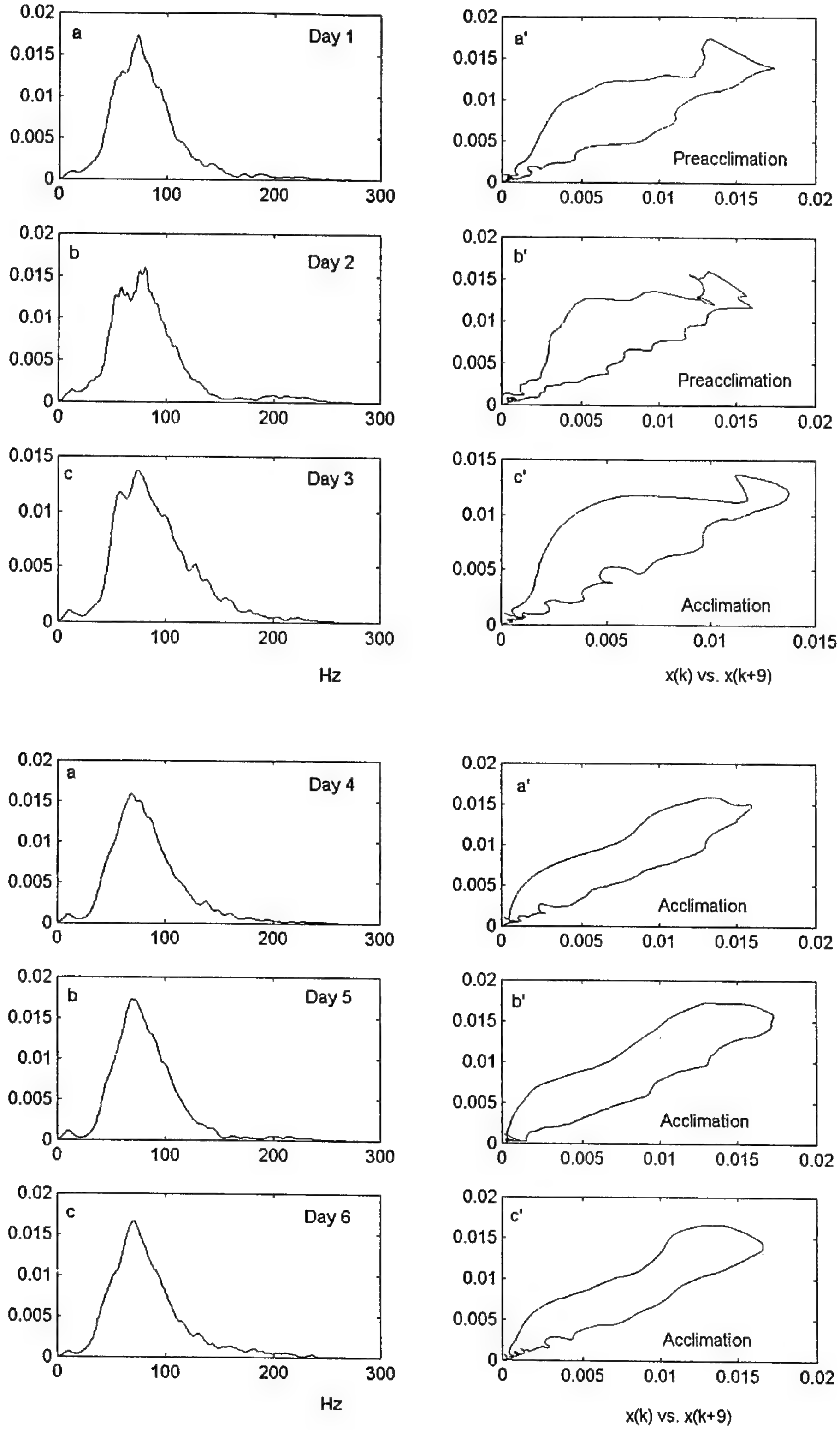

Figure 3: (a) - (f) show the spectral profiles for Rat 950059 from recorded day 1 to day 6. (a') - (f') show the corresponding state-space plots ($x(k)$ vs. $x(k+9)$).

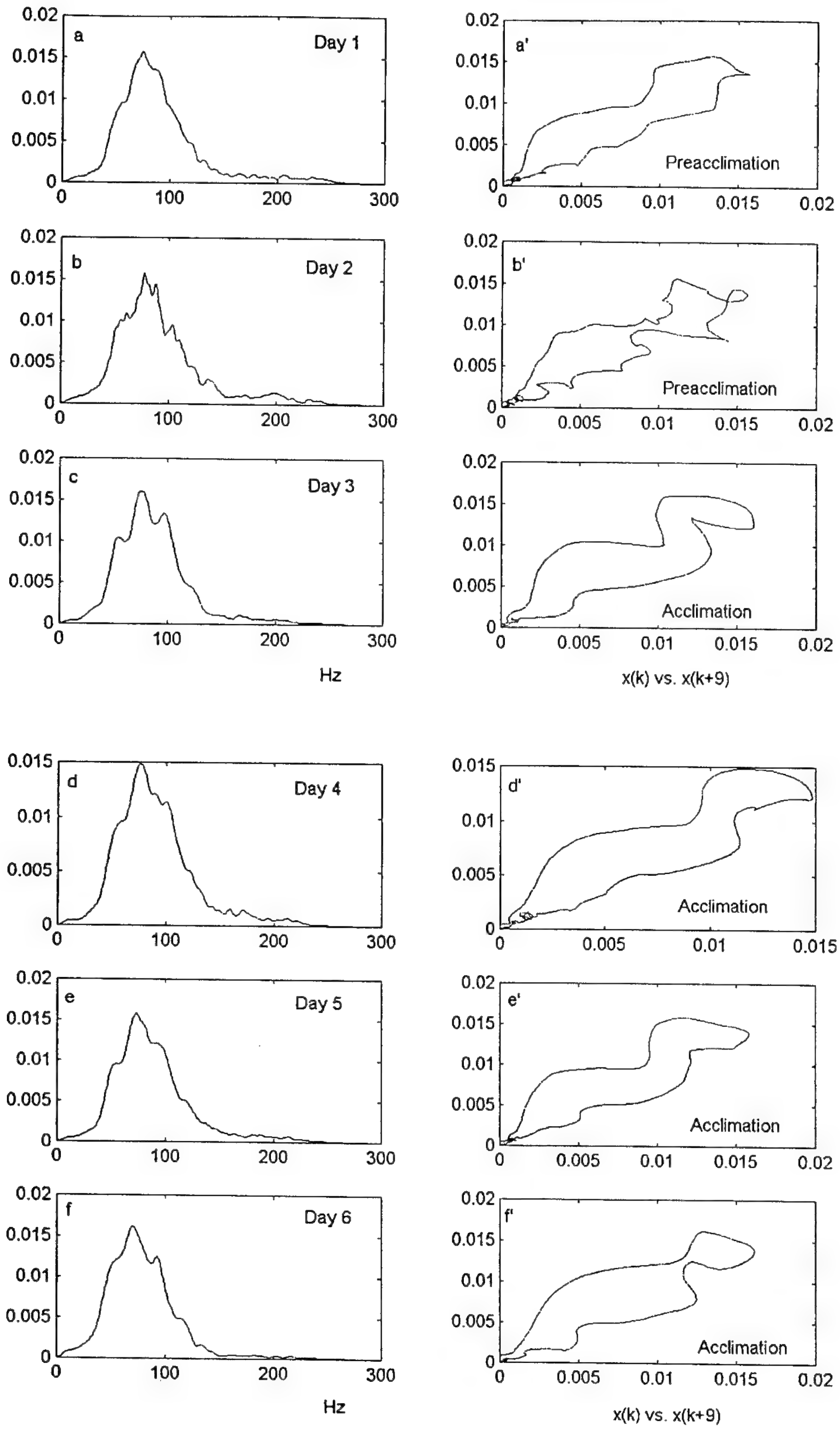

Figure 4: (a) - (f) show the spectral profiles for Rat 950158 from recorded day 1 to day 6. (a') - (f') show the corresponding state-space plots ($x(k)$ vs. $x(k+9)$).

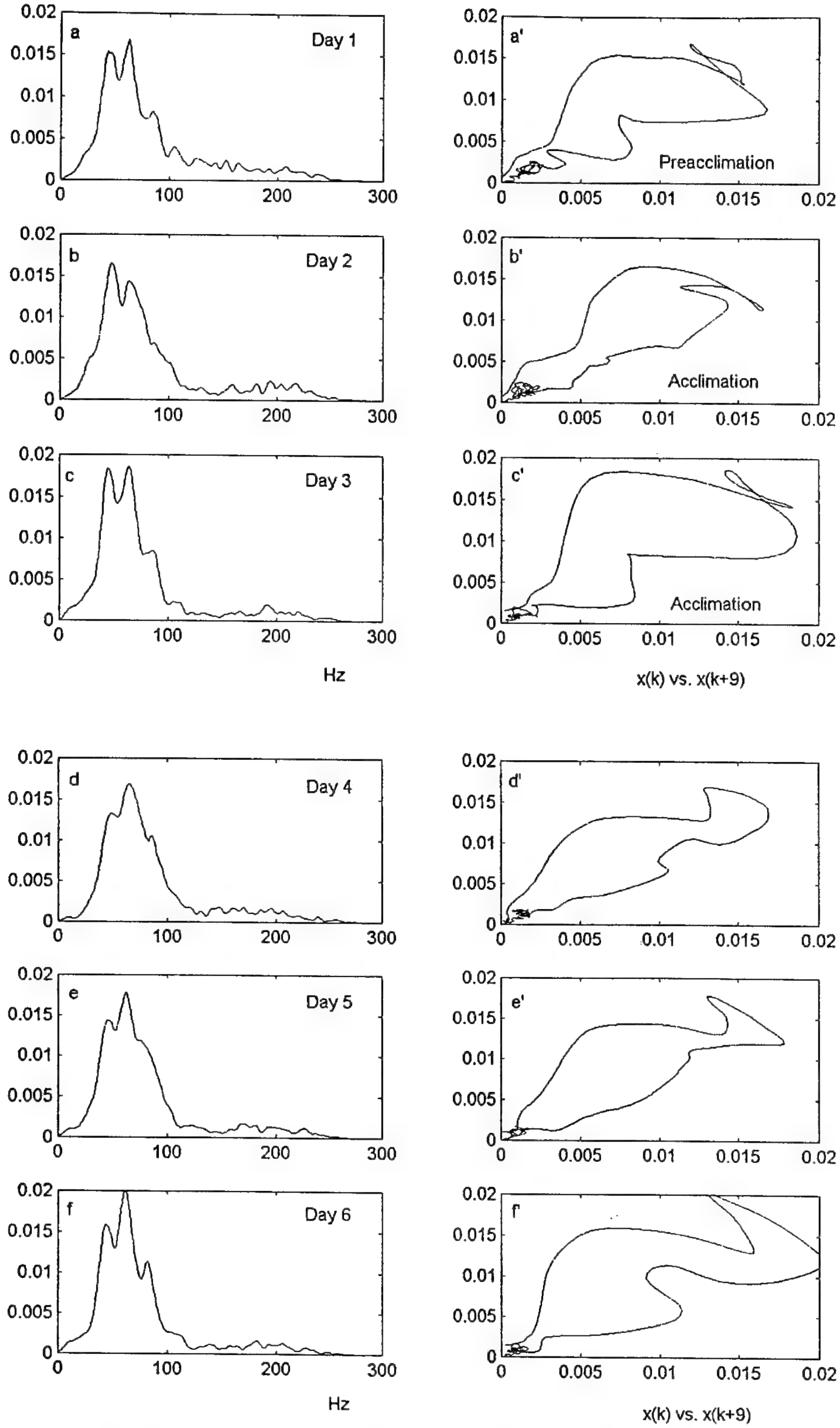

Figure 5: (a) - (f) show the spectral profiles for Rat 960017 from recorded day 1 to day 6. (a') - (f') show the corresponding state-space plots ($x(k)$ vs. $x(k+9)$).

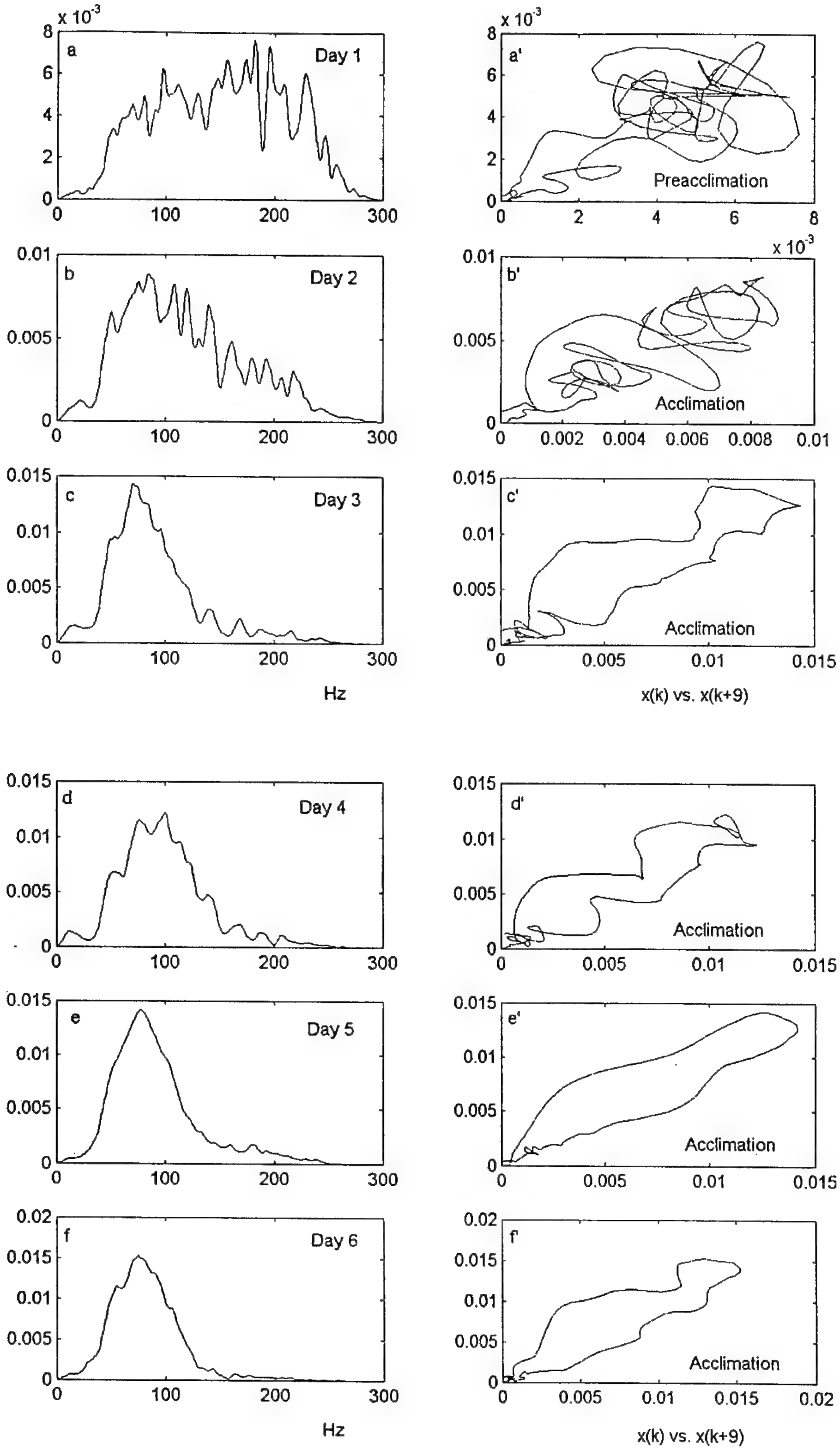

Figure 6: (a) - (f) show the spectral profiles for Rat 960107 from recorded day 1 to day 6. (a') - (f') show the corresponding state-space plots ($x(k)$ vs. $x(k+9)$).

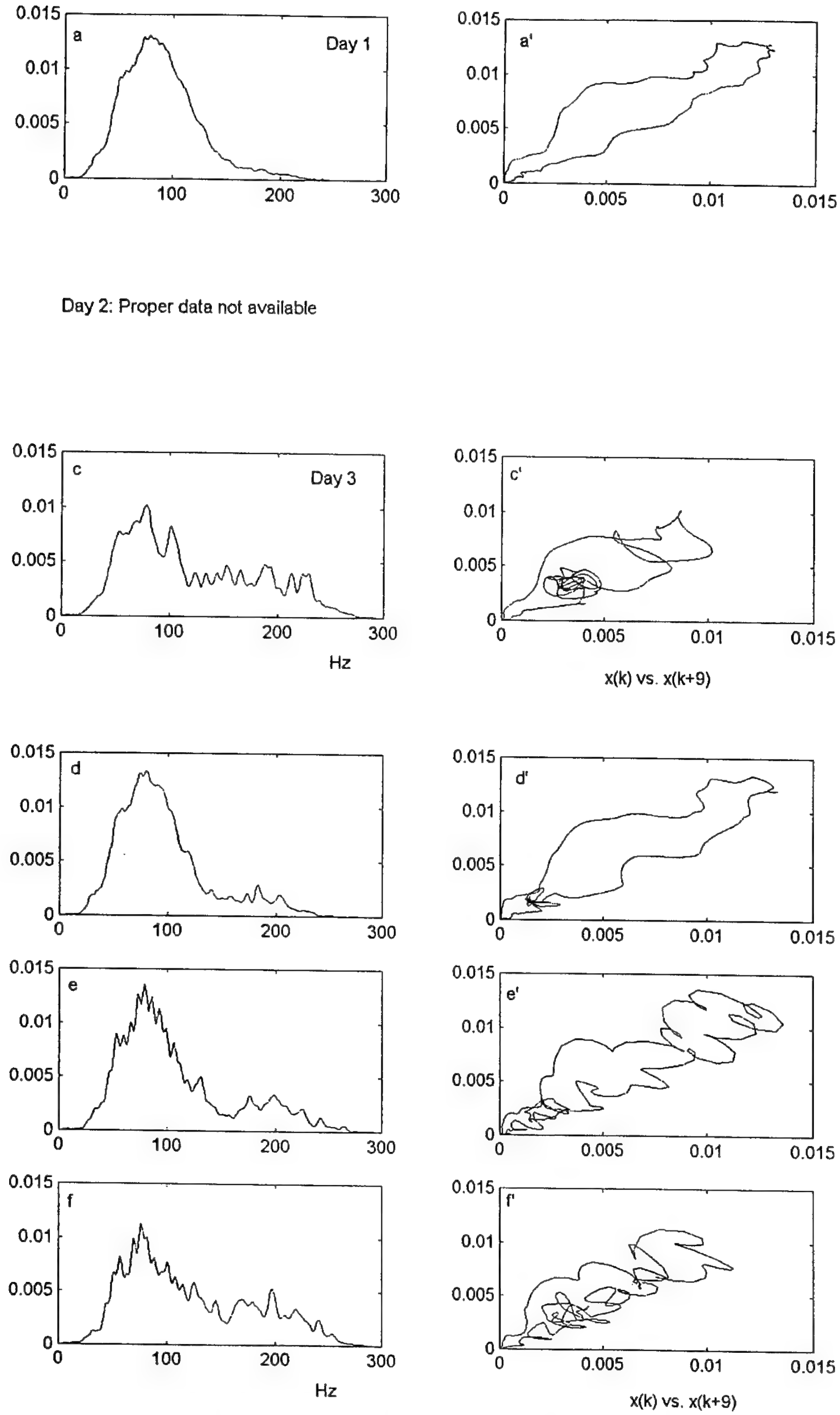

Figure 7: (a),(c), (d) - (f): Spectral profiles for Control Rat 950067 from day 1 to day 6. (a'),(c'), (d') – (f') show the corresponding state-space plots ($x(k)$ vs. $x(k+9)$). Data for recorded day 2 was not usable.

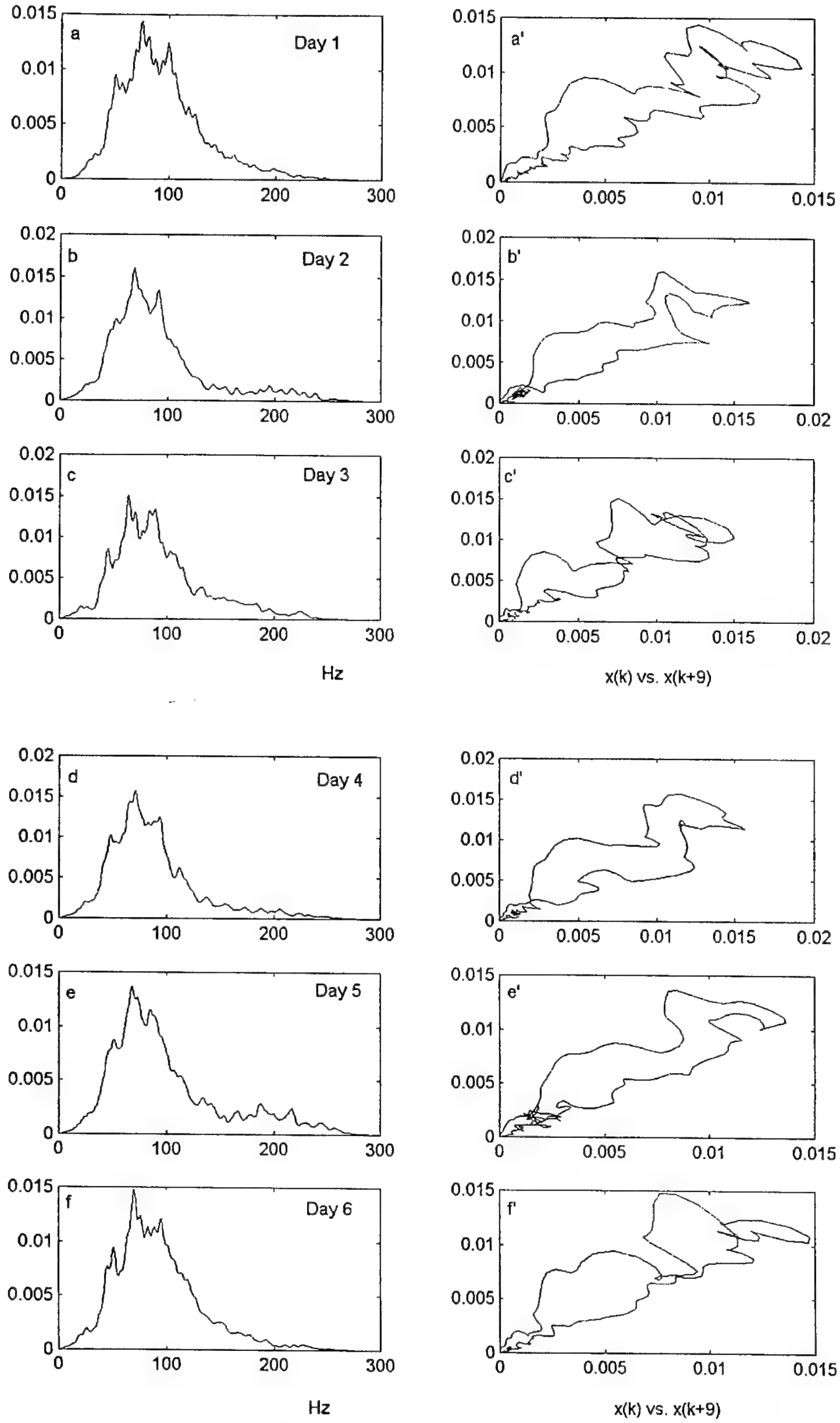

Figure 8: (a) - (f): Spectral profiles for Control Rat 950160 from recorded day 1 to day 6. (a') - (f') show the corresponding state-space plots ($x(k)$ vs. $x(k+9)$).

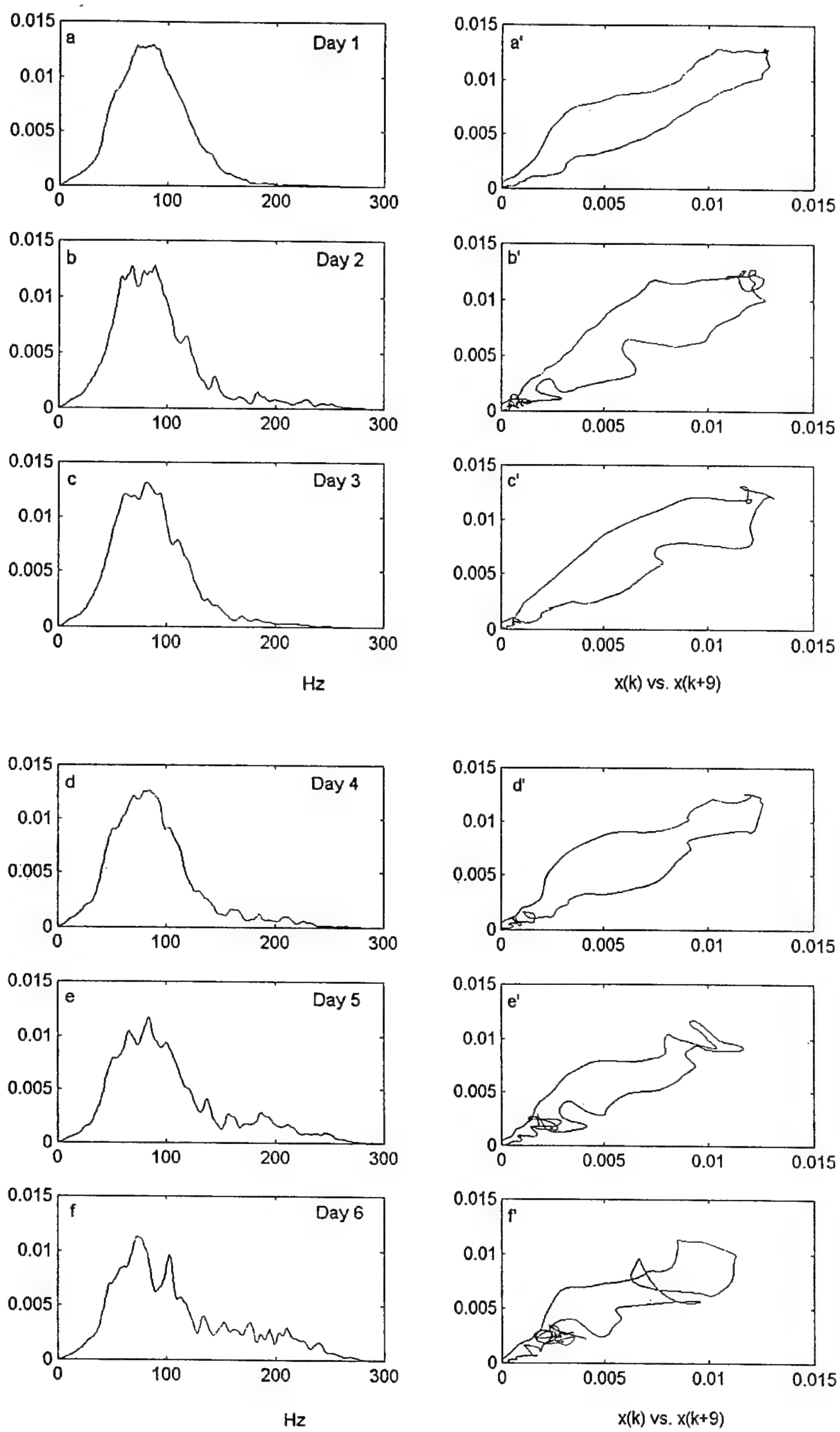

Figure 9: (a) - (f): Spectral profiles for Control Rat 960015 from recorded day 1 to day 6. (a') - (f') show the corresponding state-space plots ($x(k)$ vs. $x(k+9)$).

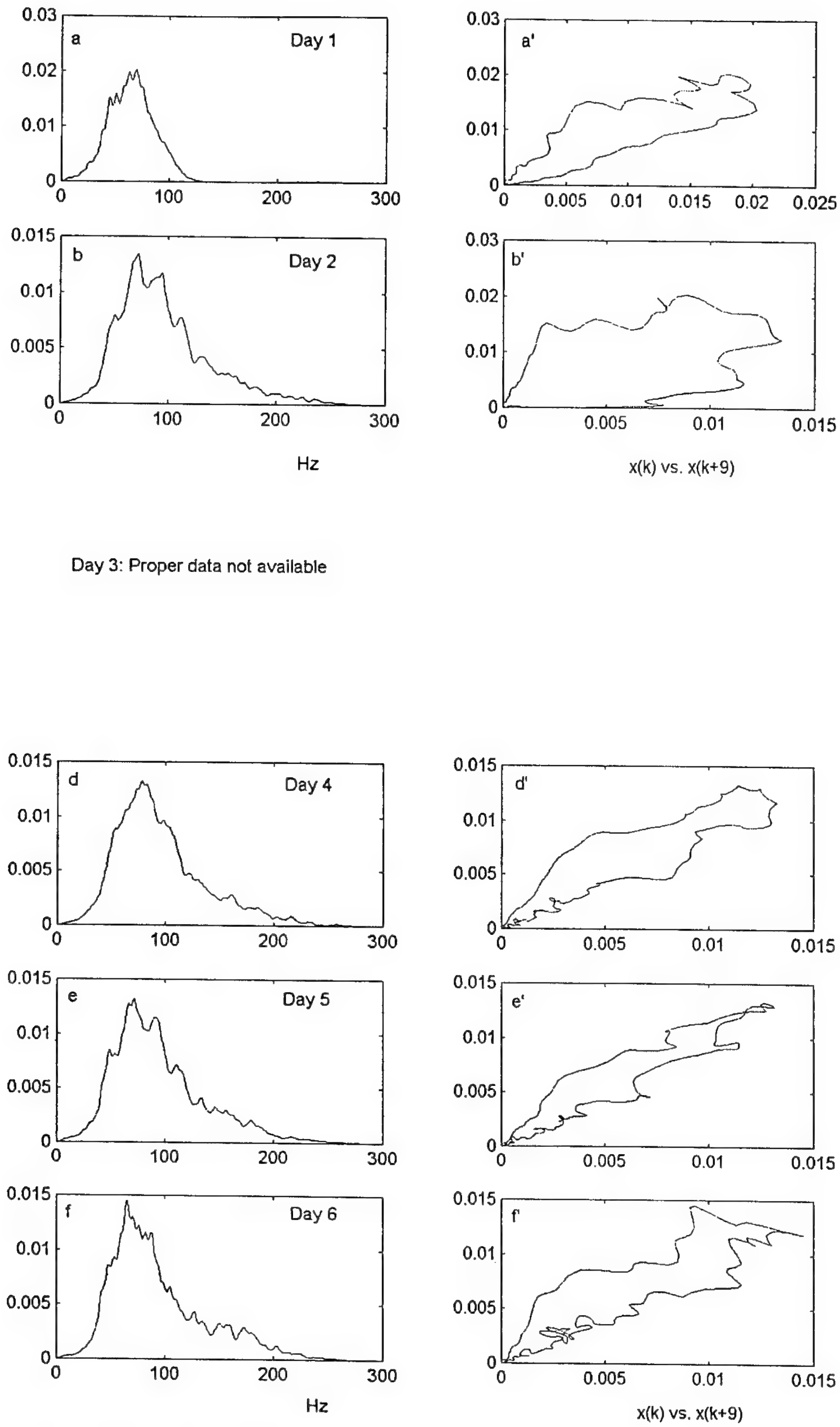

Figure 10: (a),(b), (d) - (f): Spectral profiles for Control Rat 960033 from day 1 to day 6. (a'),(b'), (d') – (f') show the corresponding state-space plots ($x(k)$ vs. $x(k+9)$). Data for recorded day 2 was not usable.

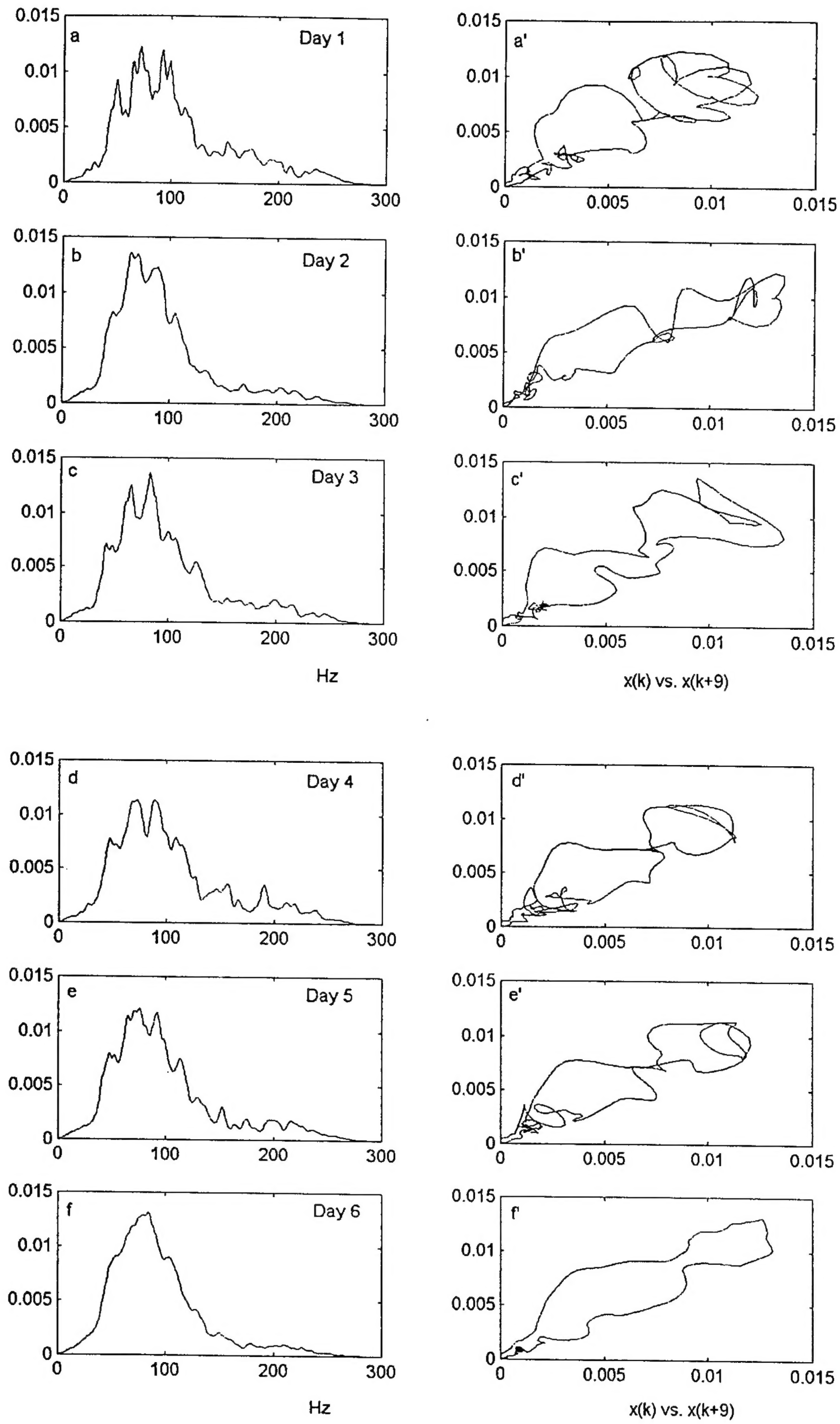

Figure 11: (a) - (f): Spectral profiles for Control Rat 960093 from recorded day 1 to day 6. (a') - (f') show the corresponding state-space plots ($x(k)$ vs. $x(k+9)$).

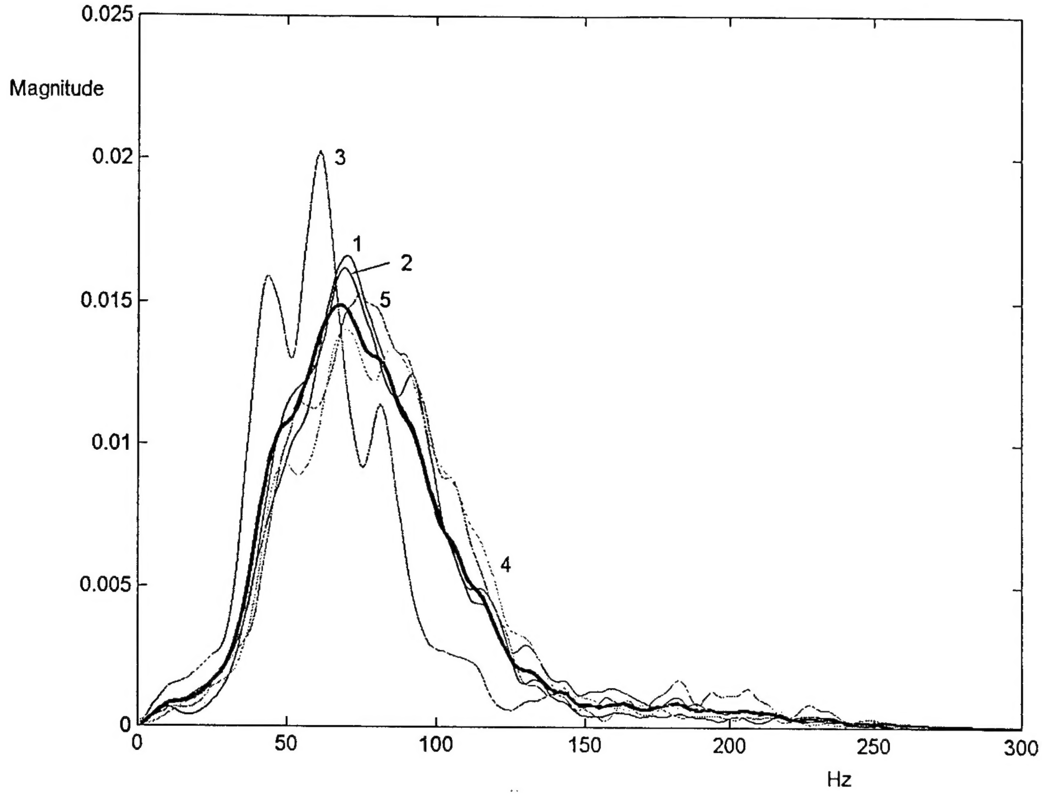

Figure 12: The spectral profiles for the sixth day for the acclimation rats and the average energy pattern (in bold). The individual profiles referred as: 1- rat 950059, 2- rat 950158, 3- rat 960017, 4- rat 960034, and 5- rat 960107.

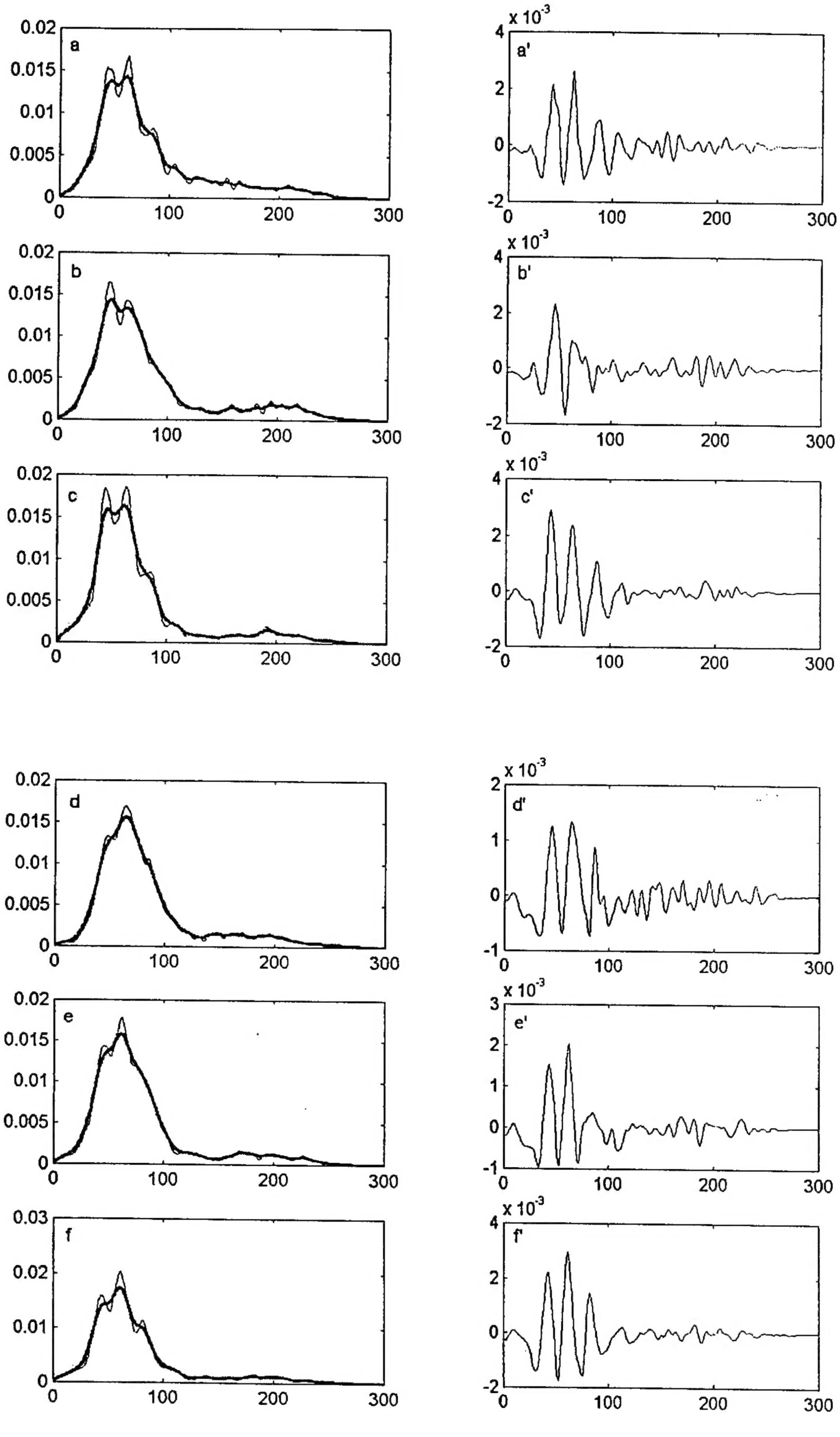

Figure 13 (a) - (f): Normal and bi-directionally filtered spectral profiles (in bold) for acclimation Rat 960017 from recorded day 1 to day 6.

(a') - (f') show the corresponding differences between the normal and the filtered profiles.

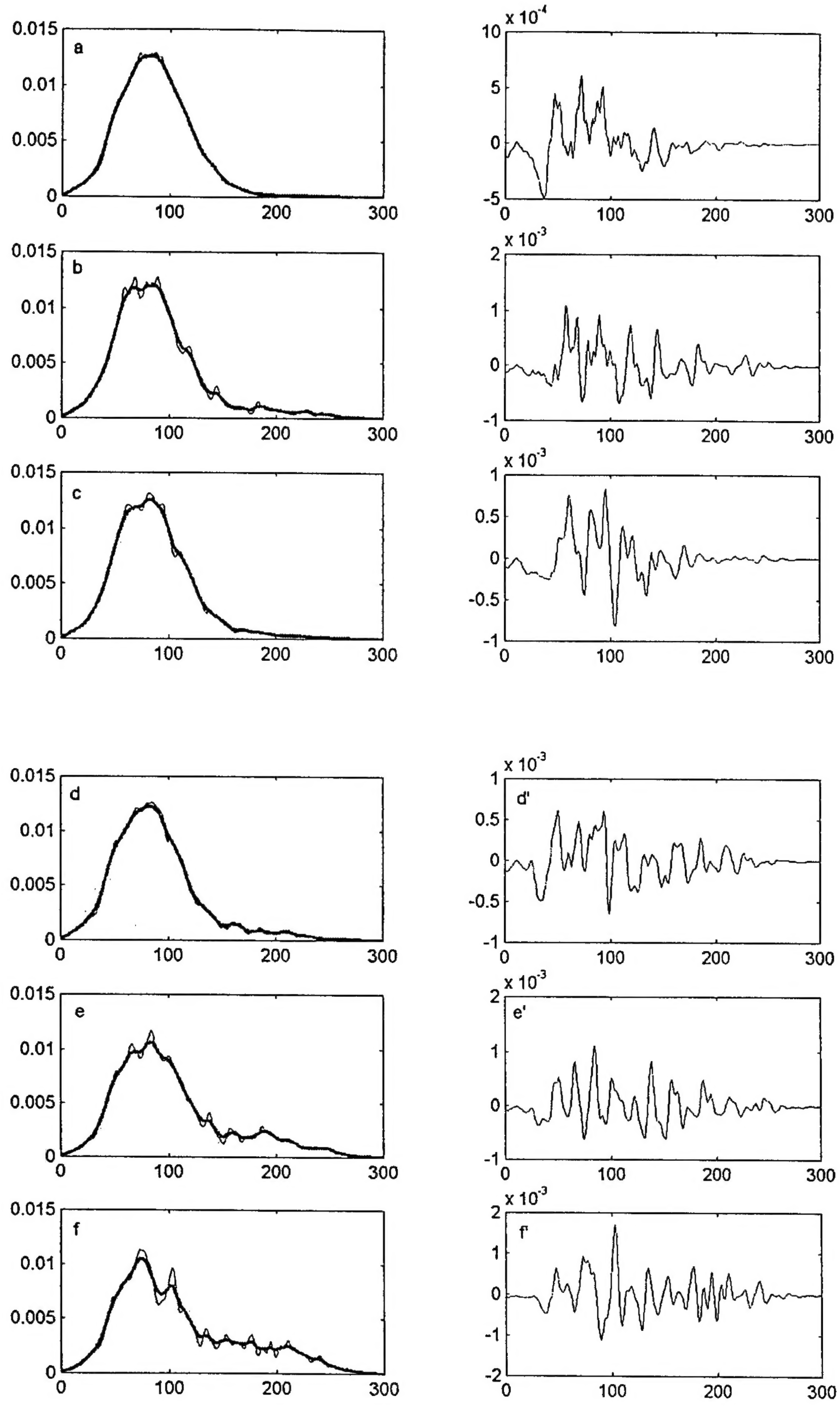

Figure 14 (a) - (f): Normal and bidirectionally filtered spectral profiles (in bold) for control rat 960015 from recorded day 1 to day 6.

(a') - (f') show the corresponding differences between the normal and the filtered profiles.